1.129(a). The appropriate Petition (with Deposit Account authorization) is therefore included in duplicate.

Remarks

Applicants acknowledge the Examiner's observation that two claims were inadvertently numbered as "Claim 188". Applicants understand that the Examiner has renumbered these claims as Nos. 188 and 189, and that all claims that follow new No. 189 (and any internal dependencies affected thereby) may be considered renumbered as of this time. If referred to below, such claims will recite the old and new numbering.

Applicants do not understand the Examiner's remarks at page 2, lines 20-24 of the pending Action to the effect that "CFTR" is unclear usage and that the "same acronym" represents "different products". CFTR stands for the protein well recognized in the art as "cystic fibrosis transmembrane conductance regulator", and is defined as such at, for example, page 1, line 31 of the Specification. Applicants have reviewed the claims and believe that there is no confusing language therein that, for example, would make it unclear as to whether the protein or an encoding DNA therefor was being referred to. Applicants also believe that usage of "CFTR" is consistent within the application. Similarly, Applicants do not understand the Examiner's position that it is improper to refer to a DNA as "including" an encoding sequence (Official Action at page 5, line 10). The requirement imposed on Applicants by law is that the language chosen be clear, and it is believed that the claims - as drafted - are clear to those skilled in the relevant art. Similarly, the Examiner's reference to "subnucleotides" (page 5, line 11) is not understood.

Applicants do not, however, assert that any particular grammatical construction they have selected is required, and the Examiner's further guidance in this respect is respectfully requested.

Applicants understand the central argument made by the Examiner in the pending Official Action to be that Applicant's Specification discloses only a few species of modified cDNA in which the cryptic promoter is inactivated (or its effects mitigated). Accordingly, the Examiner has taken the position that the Specification is non-enabling (35 USC §112, first paragraph) for the scope of the claim coverage requested(i.e. undue experimentation is required), and that therefore the allowable subject matter must be limited to the particular species described (see, for example, the Official Action at page 3, lines 9-13 citing MPEP § 706.03 (n), (z). Applicants respectfully traverse the rejection.

The relevant standard under Section 112 by which the sufficiency of Applicants' disclosure is to be measured is described, for example, in In re Wands 8 USPQ 2d 1400 (Fed Cir 1988), citing with approval Ex parte Forman 230 USPQ 546 (Board of Patent Appeals and Interferences 1986); and, for example, Atlas Powder Co. v. E.I. Du Pont De Nemours & Co. 224 USPQ 409 (Fed Cir 1984).

The Enabling Nature of Applicants' Specification has been Demonstrated

As described in detail in the Declarations "A" and "D" of Dr. Richard J. Gregory submitted June 9, 1995, authors/inventors having commonality with J. Riordan et al., "Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA", Science, 245, 1989, pp. 1066-1073 (Riordan et al. 1989) had failed to disclose the isolation of a clone encoding full length CFTR, or the construction thereof. Those inventors/authors were unaware that a cryptic RNA polymerase promoter found in the CFTR cDNA, capable of being active in E. coli, was responsible for these cloning difficulties. In

fact, certain of those authors/inventors had specifically stated that a cryptic promoter was not responsible for the cloning difficulties [see, for example, Declaration "A" and "D" at pages 4-5 thereof, and Declaration "E" of Dr. Alan E. Smith submitted herein on August 21, 1995 at numbered paragraph 34 (A) through (I) thereof on pages 12-14], thereby teaching away from the present Applicants' discoveries.

In contrast, the Applicants herein correctly determined that a cryptic RNA polymerase promoter recognized by E. coli **was responsible** for the very great difficulties that had prevented anyone from obtaining a full length DNA coding for CFTR prior to the present invention [see, for example, aforementioned Declaration "A" at page 5; Declaration "B" of Dr. Gregory also submitted June 9, 1995 at pages 3-4 thereof; Declaration "C" of Dr. Gregory also submitted June 9, 1995 at pages 1-3 thereof; and Declaration "E" at, for example, pages 2-4 thereof under the heading "Genzyme Inventions"].

Accordingly, prior to the March 5, 1990 filing date of Applicants' 07/488,307 parent application (the earliest application to which priority is claimed herein), Dr. Gregory and his co-inventors had recognized that CFTR-encoding DNA contained a bacterial RNA polymerase promoter sequence. Additionally, Applicants had demonstrated that this promoter could be rendered harmless by placing an intron at the natural exon/intron boundary at nucleotide positions 1716/1717 in the CFTR cDNA, and by using technology involving low copy number plasmids.

Prior to the March 5, 1990 filing date of the 07/488,307 parent application, the present Applicants had also identified two sites (the first between about positions 748 and 778, and the second between about positions 908 and 936) in the CFTR cDNA that had strong homology to the recognized consensus E. coli

RNA promoter sequence. In this regard, see for example Declaration "C" at pages 2-3 and Exhibit 1 attached thereto, and Declaration "E" at pages 2-3.

Applicants then identified the precise location of the cryptic promoter that was responsible for the cloning difficulties. This identification was accomplished in a simple and straightforward fashion by constructing clones in which various small fragments of the CFTR cDNA were placed upstream from a promoter-less reporter gene, and then monitoring for transcription/translation from the reporter gene to confer resistance in the presence of antibiotic [see Declaration "D" at pages 2-3 thereof, and Figure 1 of the publication attached to that Declaration as an Exhibit, **R. J. Gregory et al.**, "Expression and Characterization of the Cystic Fibrosis Transmembrane Conductance Regulator", Nature , 347, 1990, pp. 382-386; and, for example, Declaration "E" at page 4 (at paragraph 11), page 7 (at paragraph 20), and page 10 (at paragraph 30)].

The present Applicants thus conclusively proved that the cryptic promoter that had been responsible for the cloning difficulties experienced by others working in the field was located at nucleotides 908-936 in the CFTR cDNA. These facts were then disclosed (as was the utility of the T 936 C mutation) at page 17, lines 18-22 of the 07/613,592 application filed November 15, 1990 of which the present application is a file wrapper continuation. At page 17, lines 18-22 thereof, the reader is directed to review the full text of the Gregory et al. 1990 Nature publication, which is readily understood by practitioners of the relevant art.

The Section 112 Rejections

In can thus be demonstrated that Applicants are entitled to broad claims having (1) first identified the source of the difficulties that had prevented construction or propagation of a full length CFTR-encoding DNA by others; (2) provided three generally applicable solutions in response thereto; and (3) further provided those of average skill in the art with a roadmap as to the design of any

similar solutions that they may wish to practice as equivalents of Applicants' teachings and examples.

In this regard, the Examiner's attention is respectfully directed to Ex Parte Forman (a Board decision cited with approval by the Court of Appeals for the Federal Circuit in In re Wands) in which the Board stated:

> " The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art: Ansul Co. v. Uniroyal, Inc., supra. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the Specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. The factors to be considered have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the predictability or unpredictability of the art and the breadth of the claims." 230 USPQ at 547 (certain citations omitted) (emphasis added).

It now remains to discuss the scope of Applicants' disclosure in view of the above representative standards.

Point mutations within the cryptic promoter of a CFTR-encoding DNA

As aforementioned, Applicants' Specification filed November 15, 1990 identifies the cryptic promoter within the CFTR-encoding cDNA that is responsible for the cloning difficulties as encoding nucleotide positions 908-936. Applicants disclosed (Specification at Page 17, lines 18-22) that the T936C mutation substantially inactivates the cryptic promoter such that plasmids containing the CFTR-encoding sequence could be maintained at high copy number in host E. coli cells without change in the encoded human CFTR amino acid sequence. Thus a human CFTR-encoding DNA can be propagated in E. coli without unintended and toxic transcription therein (produced from a cDNA not

containing the disclosed mutation), although translation of the correct amino acid sequence would result when the encoding DNA is placed in human cells.

Reference to the then well known (in 1989-1990) derived consensus sequence for an E. coli RNA polymerase promoter (see present Specification at page 15, line 30, and Exhibit 2, page 9, attached to Declaration "D") in comparison with Figure 1 of the aforementioned Gregory et al. 1990 Nature publication shows that nucleotide 936 satisfies the criteria as a locus for point mutations:

> (1) the nucleotide should correspond to a highly conserved position in the consensus (for example, the 3' end "T" in TATAAT, see Figure 1 in Gregory et al. 1990 and Exhibit 2 attached to Declaration "D" at page 9) so that mutation thereof would be expected to decrease substantially the likelihood that the resulting sequence would be recognized by E. coli as an RNA polymerase promoter, and
>
> (2) the mutation, because of codon degeneracy, would not alter the amino acid encoded by the affected codon (in the case of the T936C mutation both AAT and AAC code for ASN).

By applying these principles, it is but routine experimentation to inspect the 908-936 cryptic promoter with the idea of identifying other such nucleotides. For example, reference to Figure 1 of the Gregory et al. 1990 Nature publication shows that CFTR nucleotide 909 (a "T") corresponds to the second "T" in the 5' end (TTGACA) of the consensus. This "T" is the most highly conserved nucleotide in the 5' end of the consensus (see Exhibit 2 to Declaration "D" , at page 9), and corresponds to the second "T" in the CTT codon that spans nucleotides 907 to 909 of the CFTR cDNA. Mutation of the 909 "T" nucleotide to any of A, G or C would nonetheless preserve LEU as the encoded amino acid (LEU codons are CTT, CTA, CTG, CTC, and also TTA, TTG). Applicants submit that this exercise is without doubt well within the skill of those practiced in the relevant art, and accordingly Applicants are entitled to a generic claim. Should

the Examiner traverse on the idea that not all such suggested mutations would be guaranteed to work (i.e. for example, the cryptic promoter's nucleotide recognition requirements do not match precisely that of the E. coli consensus at each nucleotide thereof), then the Examiner's attention is respectfully directed to Atlas Powder Co. v. E.I. Du Pont De Nemours & Co. 224 USPQ 409 (Fed Cir 1984) at page 414 thereof where, in the context of explosive powder compositions, that Court held: "We agree with the district court's conclusion on enablement. Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is not a function of the claims to specifically exclude **** possible inoperative substances ****' (citations omitted)". Simply stated, since the Applicants herein have provided more than sufficient guidance as to how those skilled in the art could further proceed, it cannot be required of Applicants to test every possible mutation and rule out possible inoperative ones, especially since it is merely a matter of routine experimentation to identify the sites, make the mutations, and then test for any unintended bacterial expression of CFTR fragments.

Insertion of an intron downstream from the CFTR cryptic promoter

Applicants observe that arguments similar to those presented above also apply to the placement of an intron within the encoding cDNA. Introns (i.e "intervening sequences"- see the language of Claim 168) that are useful according to the practice of the invention are those capable of being spliced from CFTR primary RNA transcript when expressed in eucaryotic cells, and either disrupt the translational reading frame of the CFTR-encoding cDNA, or contain one or more stop codons, so that production in host E. coli cells of toxic CFTR fragments is minimized. The Examiner is requested to refer to page 11 of the Reply with Amendment filed herein on June 9, 1995 wherein certain phrases pertinent to the description of introns/intervening sequences useful in the practice of the

invention (for example, toxic CFTR fragments, intervening sequence, disrupts expression, spliced from CFTR primary RNA, disrupts the translational reading frame, disrupts the protein coding sequence, stop codons) were discussed, and appropriate page/line citations to the use thereof in the Specification were listed.

It is clear from a reading of the present Specification that those skilled in the art would recognize that introns ("intervening sequences") useful in the practice of the invention need to be placed downstream from the cryptic promoter (actually downstream from the initiator methionine of the promoter), but preferably close thereto, so that the length of any truncated CFTR polypeptide sequence that is nonetheless translated (owing to action of the cryptic promoter) is minimized to thereby minimize toxicity. Once the precise location (CFTR cDNA nucleotides 908-936) of the promoter was disclosed to those skilled in the art, it became but a matter of routine experimentation to select additional sites, besides the exemplified nucleotide 1716/1717 site first chosen by Applicants, for placement of an "intervening sequence".

Modifications made outside the CFTR-encoding DNA sequence

Applicants' Specification (see Examples 1 and 2 thereof; Examples 2 and 3 of the 07/488,307 parent application filed March 5, 1990) discloses that a plasmid containing a CFTR-encoding cDNA sequence can be successfully propagated in host E. coli cells if the plasmid is designed to contain an origin of replication that permits maintenance of the plasmid at only about 25 copies or less per host cell, i.e at low copy number. This aspect of Applicants' inventions involves the recognition that if the promoter itself is not inactivated, other methods must be found to minimize effectively the amount of "toxic transcription" that will otherwise result.

Applicants' use of introns and point mutations are examples of successful modifications that are made within the CFTR encoding sequence itself.

However, it is well within the knowledge of this art that effective modifications can be made in, for example, a plasmid that contains the CFTR encoding cDNA, but wherein the modification is made to the plasmid outside of the CFTR-encoding sequence itself. Applicants use of particular origins of replication is but one such example. Additional applicable techniques (well-recognized as of 1990) include (1) placement of an antisense RNA polymerase promoter at the 3' end of the CFTR-encoding cDNA to limit the amount of toxic transcript that is translated in the host E. coli cells; and (2) maintenance of the host E. coli cells in the presence of an antibiotic (such as a DNA gyrase inhibitor) that can limit the copy number of plasmids maintained therein.

Accordingly, it is demonstrated that Applicants herein have placed those skilled in the art in possession of a considerable and varied set of approaches for the propagation of CFTR-encoding DNA molecules in host E. coli cells.

Conclusion

Applicants believe that a telephonic interview will facilitate resolution of remaining issues and request that this be arranged at the Examiner's convenience.

No Request for an Extension of Time is believed to be due in connection with this Reply since it is being filed within 3 months of the mailing date of the pending Examiner's Action. An appropriate Petition for Transitional After-Final Practice (including authorization to charge **Deposit Account 07-1074**) is included herewith in duplicate. Should the Patent Office determine that any other fees are due, then the Patent Office is authorized to charge to **Deposit Account 07-1074** any claim fee, or any other fee, that it determines is necessary to secure the entry and full consideration of this Amendment.

Applicants would again like to gratefully acknowledge the Examiner's extensive efforts on behalf of this application and respectfully submit that this response completes the address of all issues. An early and favorable action is respectfully requested.

Respectfully submitted,

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Dated: January 17, 1996

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